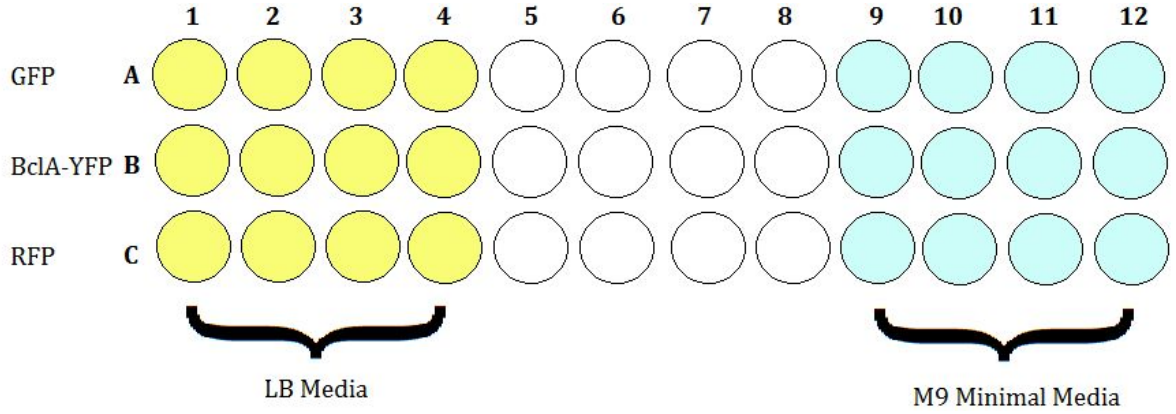
Week 2 Notebook

June 8, 2015 - June 12, 2015

June 8, 2015

Kayla/Julie

- Made M9 minimal media following protocol
- Biofilm assay
    - 4 96 well plates
        - 1 @ 25°C for 4 hrs
        - 1 @ 25°C for 24 hrs
        - 1 @ 37°C for 4 hrs
        - 1 @ 37°C for 24 hrs
    - 100 µL of 1:100 dilution in 12 wells according to the diagram below



    - dumped and rinsed wells, tapped dry onto paper towel
    - stained wells of 4 hour plates with 125 µL of 0.1% crystal violet
        - dumped out crystal violet
        - rinsed in one beaker of water and tapped out crystal violet
        - rinsed in a second beaker of water and tapped dry onto a paper towel
    - plates left to incubate at 25°C and 37°C for 4 hours produced no biofilms
- Poured plain LB plates
- Picked 3 colonies from 200 µL GFP+J23109 and CFP+J23109 plates and prepared 5 mL liquid cultures

Chloe/Charlotte

Finalized design for TouchTomorrow poster on DNA and general synthetic biology poster. Prepared test freezing survival assay using cultures of *E. coli* containing inserts 14-34C (YFP), 14-35C (GFP), and RFP from transformation test kit.

- First, using cultures in the stationary phase
    - 10-fold serial dilutions, beginning with 100 ul culture and 900 ul LB + Cam
    - Plated 100 ul of the 10^{-5}, 10^{-6}, and 10^{-7} dilutions and placed in incubator.

    - 1 ml of culture each was placed in -20C and -80C temperatures.
    - Dilution and plating procedure will be repeated with the -20C and -80C samples after overnight incubation.
- Using cultures in the log growth phase
    - a 1:20 dilution of each stationary culture was used (250 ul of culture into 5 ml of LB and 5 ul of Cam)
    - Placed in shaker to grow for 2 hours; OD measured at ~0.76 at 690 nm
    - 1:10 dilution was made and OD measured at ~0.27 after 45 minutes
    - grown again and measured at ~0.5 after 45 more minutes
    - 10-fold serial dilutions, beginning with 100 ul culture and 900 ul LB + Cam
    - Plated 100 ul of the 10^{-5}, 10^{-6}, and 10^{-7} dilutions and placed in incubator.
    - 1 ml of culture each was placed in -20C and -80C temperatures.
    - Dilution and plating procedure will be repeated with the -20C and -80C samples after overnight incubation.

Dave/Eddie

- Measured the absorbance of digested J23108, J23111, 15-5A and 15-6C:

Substance	Absorbance @260	Absorbance @280	Concentration (ug/ul)
J23108 (15-8C)	0.0216	0.0161	1080
J23111 (15-9C)	0.0209	0.0158	1045
15-5A	0.0235	0.0184	1175
15-6C	0.0197	0.0148	985

- We are cloning GFP and CFP into J23111 and J23108
    - J23111 = 15-9C
    - J23108 = 15-8C
    - CFP = 15-6C
    - GFP = 15-5A
- The first step is to digest the vectors with primers using SpeI and PstI, conditions to follow:

Volume	Ingredient	
5 ug DNA (~5ul)	15-9C	15-8C
2 ul	Spe	“
2 ul	Pst	“
2 ul	Buffer	“

qs to 20 ul (11 ul)	H2O	“

- 5 ul DNA used for both reactions
- 11 ul H2O used

- The gel displayed insufficient DNA to cut a band, it is unknown what went wrong. We will make another attempt tomorrow.
  - We need to use the original miniprep, not the digest that we did use
- Also to clone tomorrow:
  - J23100 + ZeAFP
    - miniprep of J23100 should exist in 2014 box, will probably need to make a new miniprep, there may be a glycerol stock, if yes, streak a plate.
    - if not, do another transformation of 14-5A, then miniprep, digest 5ug (same for ZeAFP) with Spe and Pst and CIP, gel purify, should release an RFP as well as the plasmid, we want the plasmid part.
    - Cut ZeAFP 15-4C with Xba and Pst, dont CIP the insert, just the vector
    - ligate them together

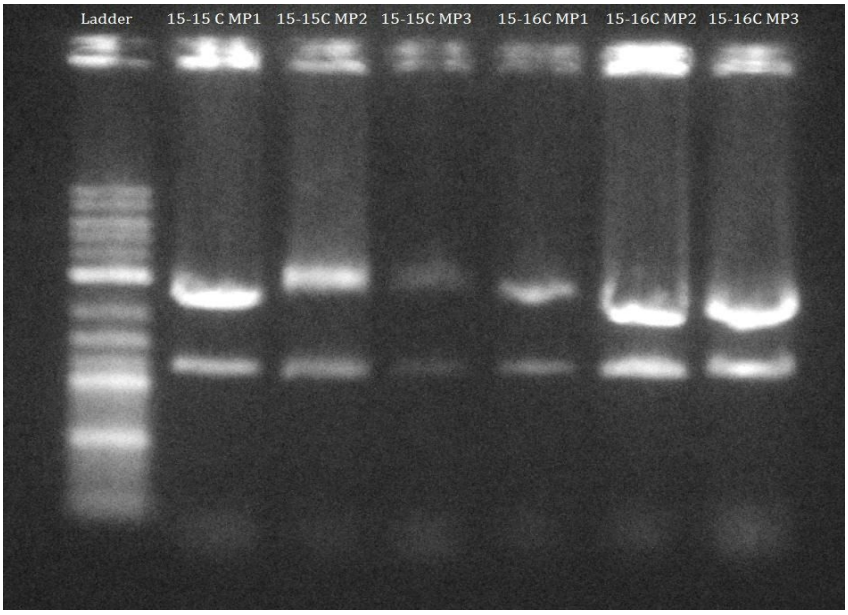
June 9, 2015

Kayla/Julie

- Miniprep of GFP and CFP with J23109 (now 15-15C and 15-16C)

Sample	Abs_{260nm}	[DNA]
15-15C Miniprep 1	0.0516	2.58 µg/mL
15-15C Miniprep 2	0.0444	2.22 µg/mL
15-15C Miniprep 3	0.0379	1.90 µg/mL
15-16C Miniprep 1	0.0184	0.92 µg/mL
15-16C Miniprep 2	0.0323	1.62 µg/mL
15-16C Miniprep 3	0.0485	2.43 µg/mL

- Test digests of GFP and CFP with EcoRI and PstI
  - 5 µL DNA, 1 µL EcoRI, 1 µL PstI, 5 µL buffer, dH_20 to 50 µL total volume
  - ran on 1% agarose gel
  - Each miniprep appeared to have the correct insert



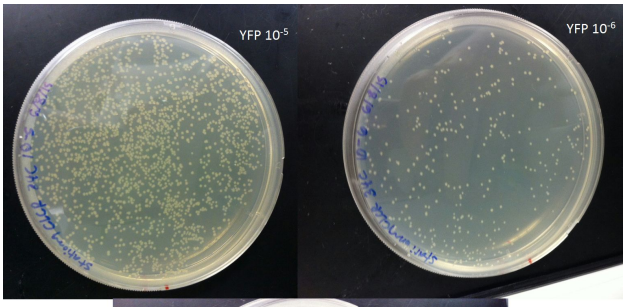
- Biofilm assay
    - stained wells of 24 hour plates with 125 µL of 0.1% crystal violet
        - dumped out crystal violet
        - rinsed in one beaker of water and tapped out crystal violet
        - rinsed in a second beaker of water and tapped dry onto a paper towel
    - no biofilms on the overnight 25ºC plate or the overnight 37ºC plate
- Prepared 17 500 µL aliquots of 1000x L-Arginine
- Prepared liquid cultures for 15-15C, 15-16C, DH5 alpha, and two biofilm forming strains
    - 15-15C= 3 0.5 mL cultures with Cam
    - 15-16C= 3 0.5 mL cultures with Cam
    - Biofilm assay strains= 3 5 mL cultures in plain LB

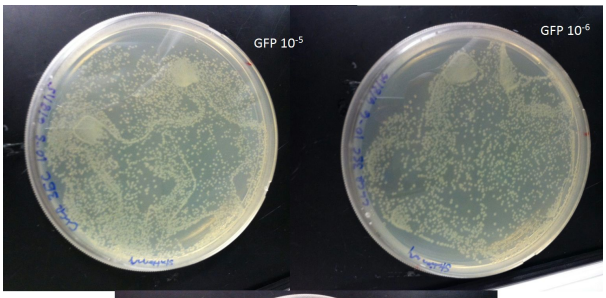
Chloe/Charlotte

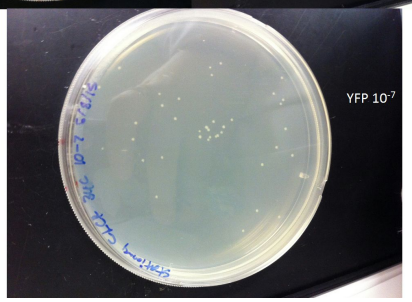
Removed first set of freeze survival test plates from the incubator and counted colonies present on each plate. The log phase growth plates were left to incubate, since they had had less time to incubate overall than the stationary phase plates. Some stationary plates had colonies counted in full and some (34C 10-5, 35C 10-5, 35C 10-6, 35C 10-7, RFP 10-5, and RFP 10-6) had colonies counted in one quadrant of the plate and multiplied by four due to very high numbers of colonies on the plate. The numbers of colonies on each plate for the stationary phase *E. coli* are as follows:

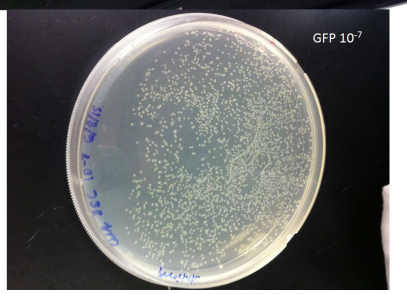
Colonies of Stationary Phase Growth *E. coli* Plates (Before Freeze)					
Strain & Dilution	Number of Colonies	Strain & Dilution	Number of Colonies	Strain & Dilution	Number of Colonies

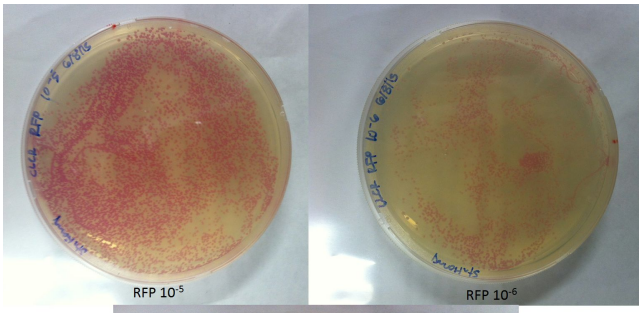
34C 10-5	1956	35C 10-5	4204	RFP 10-5	4084
34C 10-6	307	35C 10-6	2824	RFP 10-6	2228
34C 10-7	38	35C 10-7	2840	RFP 10-7	947

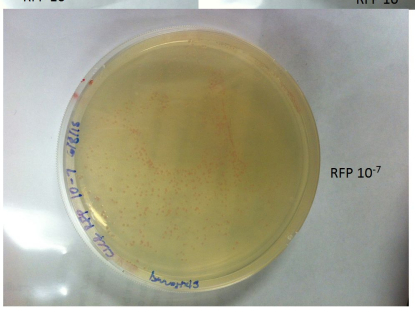












The log phase growth plates were taken out of the incubator after being left to incubate for an additional 5 hours. However, there was not enough time to count the colonies, so the plates were wrapped in plastic wrap and placed in the 4°C fridge to rest without growth overnight until the next day when they were to be counted.

2 liters of LB media were prepared to pour more chloramphenicol plates

Began a test transformation of the competent cells using the 35C plasmid. Tested against the competent cells purchased from NEB.

- Untransformed cells of each type also put through the protocol to determine survival.
- 50 ul cells and 1 ul DNA placed in a 1.5 ml tube and incubated on ice for 30 min
- All cells heat shocked at 42℃ for 1 minute, then left to incubate for 5 minutes on ice.
- 200 uL SOC media added to each tube, tubes left to incubate in a 37℃ shaker for one hour.
- 250 uLs of each culture plated (LB plates for untransformed cells, LB+Cam plates for transformed cells)

Once LB+Cam plates were set, dilutions up to 10-7 were made of the cultures left in the freezer for the freeze survival test. 12 dilutions were made in total, for RFP, GFP, and YFP at both stationary and log phase growth and at both -20℃ and -80℃.
The 10-5, 10-6, and 10-7 dilutions were plated and left to incubate overnight.

Dave/Eddie

- We are redoing the digest from yesterday using the undigested DNA instead. We used the following digest conditions at 37C for 1 hr.

Ingredient	Volume	
5 ug DNA	15-9C 5ul	15-8C 14 ul
Buffer	2 ul	2 ul
SpeI	2 ul	2 ul
PstI	2 ul	2 ul
qs to 20 ul w/ H2O	9 ul	0 ul

- We also digested 14-5A and 15-4C

Ingredient	Volume	Ingredient	Volume
14-5A	5 ul	15-4C	5 ul
Cutsmart Buffer	2 ul	Cutsmart Buffer	2 ul
Spe	2 ul	XbaI	2 ul
Pst	2 ul	Pst	2 ul
qs to 20 H2O	9 ul	qs to 20 H2O	9 ul

Total	20 ul	Total	20 ul

- Everything will be treated with CIP for 20 min at 37C **except 15-4C, the ZeAFP because it is an insert**
    - CIP treatment - addition of
    - 27 ul H2O
    - 3 ul buffer
    - 1 ul CIP
    - Incubate 37C for 20 min

- Samples were run on a gel with the following wells:
    1. Ladder
    2. 15-4C
    3. 14-5A
    4. 14-5A
    5. 15-8C
    6. 15-8C
    7. 15-9C
    8. 15-9C

- Gel extracted wells 6, 7, 8. Well 5 was suspect, and was not kept. Wells 2-4 did not contain sufficient DNA to extract. See DM ED gel 6-9-15.
- Gel Extract Results

Substance	Absorbance @260	Absorbance @280	Concentration (ug/ul)
15-8C	0.0034	0.0029	170
15-9C	0.0051	0.0044	255

- Picked three colonies of J23108 to grow overnight.
- 14-5A will not be used, we will instead be using J23119 (15-17C).
    - we will need to pick and transform J23119
    - Today: get it from the plate, transform, plate it
    - Tomorrow: pick a colony, begin a miniprep
    - Thur: glycerol stock, miniprep
- We picked J23119 from 2015 kit plate 3 well 17O
- Transformed 2 ul DNA with 50 ul competent *E. coli*
- Incubated on ice 30 min, heat shock 42C 1 min, incubate on ice 5 min, add 200ul SOC, incubate 37C shaking 1 hr, plate 50 ul and 200 ul on Cam plates, incubate overnight
- The concentrations of 15-4C, 15-5A, 15-6C, 15-8C, and 15-9C were found using a spectrophotometer:

Substance	Absorbance @260	Absorbance @280	Concentration (ug/ul)

15-4C	0.0102	0.0076	510
15-5A	0.0030	0.0014	150
15-6C	0.0044	0.0021	220
15-8C	0.0034	0.0029	170
15-9C	0.0051	0.0044	255

- Ligation Table

Ingredient	**15-8C** + *15-6C*	**15-8C** + *15-5A*	**15-9C** + *15-6C*	**15-9C** + *15-5A*
Buffer (ul)	2	2	2	2
Ligase (ul)	1	1	1	1
Backbone (ul)	3	3	3	3
Insert (ul)	3	3	3	3
H2O	11	11	11	11
Total (ul)	20	20	20	20

- Ligations left at RT overnight
- 3 15-4C colonies were picked and placed in LB broth with CAM in the shaker overnight in preparation for a glycerol stock and miniprep to be made. This was done because we were running low on the purified plasmid.
- 1L CAM LB agar was made into 17 square plates, 20 small round plates, and 7 standard petri dishes.
- We redid the digest of 15-4C with XbaI and PstI in a 50ul reaction and left it in the freezer overnight to gel purify tomorrow.

Digest Table

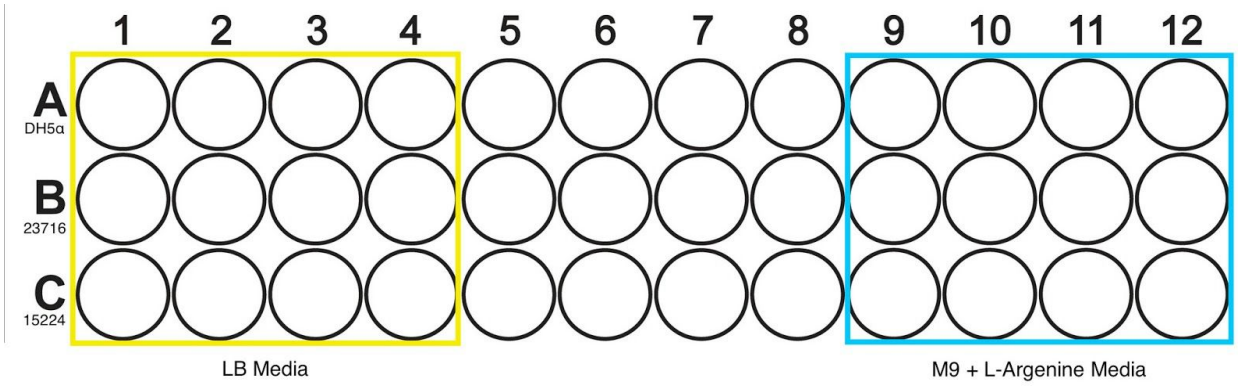
Ingredient	Volume
15-4C (510 ug/ul)	10 ul
Cutsmart Buffer	5 ul
XbaI	2 ul
Pst	2 ul
qs to 50 H2O	31 ul

Total	50 ul

June 10, 2015

Kayla/Julie

- Made glycerol stocks of 15-15C and 15-16C
- Biofilm assay
    - 4 96 well plates
        - 1 @ 25°C for 48 hrs
        - 1 @ 25°C for 24 hrs
        - 1 @ 37°C for 48 hrs
        - 1 @ 37°C for 24 hrs
    - 100 µL of 1:100 dilution in 12 wells according to the diagram below

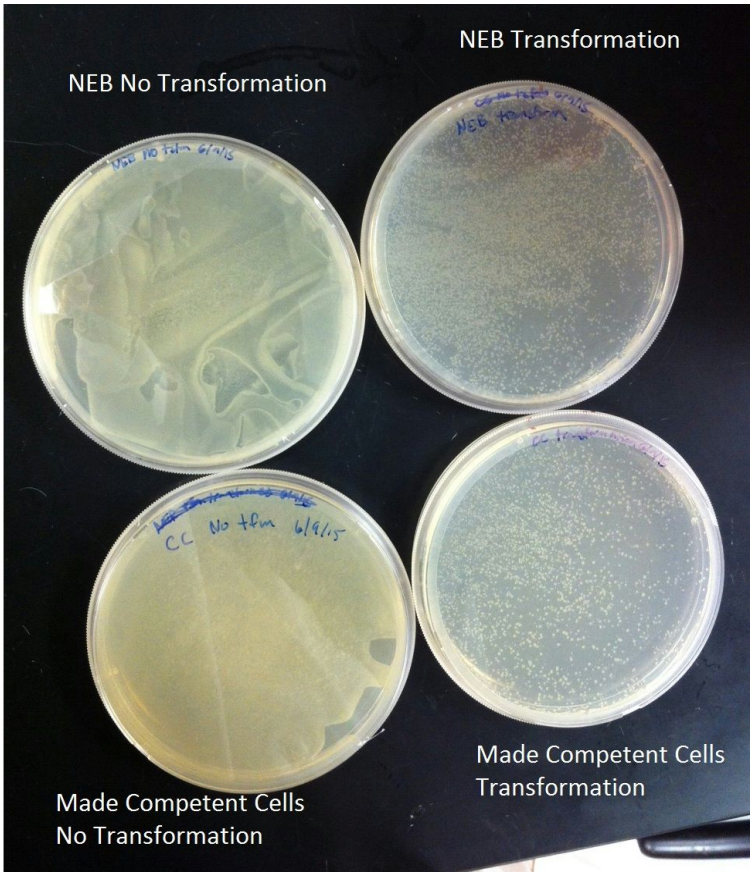


- Prepared samples of 15-15C for sequencing
- Poured LB + Cam plates

Chloe/Charlotte

Unfortunately, the incubator did not get hot enough to allow the freeze survival test plates to grow efficiently, only reaching 30°C. The incubator was turned up and the plates were incubate for an additional day.

Photographs were taken of the test transformation plates, and the test was deemed a success. The home-made competent *E. coli* transformed and grew just as efficiently as the competent cells purchased from NEB.



As there was not enough time to count pre-freeze log phase growth colonies for the freeze survival test yesterday, the plates were placed in the fridge at the end of the day to rest without growth at 4℃. At the beginning of the day, the plates were removed from the fridge and colonies were counted.

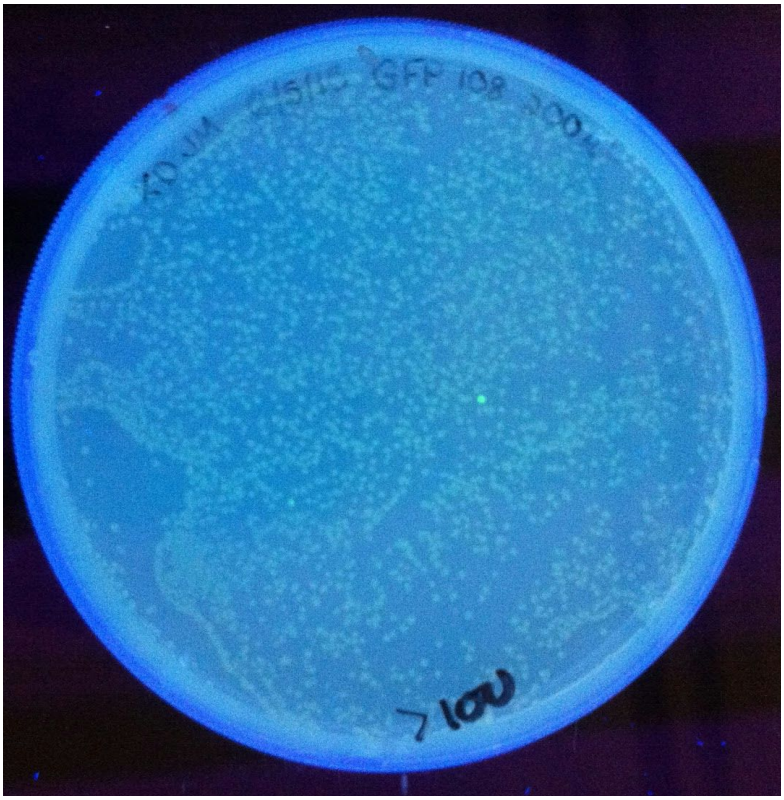
The pre-freeze log phase growth plates did not have numbers of colonies as high as the pre-freeze stationary phase growth plates, so all colonies were counted in full. The numbers of colonies on each plate for the log phase *E. coli* are as follows:

Colonies of Log Phase Growth *E. coli* Plates (Before Freeze)					
Strain & Dilution	Number of Colonies	Strain & Dilution	Number of Colonies	Strain & Dilution	Number of Colonies
34C 10-5	143	35C 10-5	567	RFP 10-5	394
34C 10-6	255	35C 10-6	356	RFP 10-6	138
34C 10-7	78	35C 10-7	214	RFP 10-7	33

5 ml liquid cultures of 14-34C, 14-35C, 15-13C, 15-14C, 15-15C, and 15-16C were prepared from glycerol stocks (#1 for 15-15C and #2 for 15-16C), in preparation for TouchTomorrow. A culture of the single glowing colony from a GFP plate was also prepared.

- New glycerol stocks should maybe be prepared for the 15-XX bacteria; it's possible the existing stocks were left out too long.

Every plate that has been made thus far was examined under the UV light. It was discovered that the vast majority of colonies of nearly every fluorescent construct created so far does not contain the correct insert. However, between all the plates, one colony was found to glow under UV light. This colony without a doubt is expressing GFP+J23108.



Dave/Eddie

- We picked 3 colonies from the 50 ul plate of 15-17C and put them each in 5 ml LB with 5 ul CAM and left them to incubate overnight in the 37C shaker.
- We transformed the 15-8C and 15-9C ligations using 5 ul of DNA and 50 ul of competent cells.
- We gel purified the digested 15-4C. The ZeAFP appears to have not been cut out of the plasmid, we will have to try again. The gel again ran weird, well 3 ran differently than well 2, though both were from the same tube.
    - Wells:
    - 1. Ladder
    - 2. 15-4C
    - 3. 15-4C

- We will try again using the plasmids purified from the miniprep conducted today.
- We conducted a miniprep of 15-8C and 15-4C that had been cultured overnight according to protocol.
- We ran out of CAM plates, more will need to be prepared today. 2L of LB agar were made.
- We will attempt the digest of ZeAFP, 15-4C. As a precaution all three samples prepared will be tested. We will also run an uncut plasmid control on the gel when extracting.
- Spectrophotometer Data

Substance	Absorbance @260	Absorbance @280	Concentration (ug/ul)
15-8C 1	0.0023	0.0017	115
15-8C 2	0.0051	0.0046	255
15-8C 3	0.0047	0.0044	235
15-4C 1	0.0096	0.0079	480
15-4C 2	0.0089	0.0076	445
15-4C 3	0.0077	0.0067	385

- Digestion Table

Ingredient	15-4C 1	15-4C 2	15-4C 3
DNA 5 ug	10 ul	11 ul	13 ul
Cutsmart 10x buffer	5 ul	5 ul	5 ul
Pst	2 ul	2 ul	2 ul
Xba	2 ul	2 ul	2 ul
qs to 50 ul	31	30	28
Total	50	50	50

- The fragment we want, the actual ZeAFP, is small, 282 bp long. To give us the best chance of properly extracting, we will increase the gel concentration to 1.5% agarose gel rather than 0.9%. We will also add additional SYBR green to the sample as the small size prohibits intercalation of the dye.

June 11, 2015

Kayla/Julie

- Prepared chlorenphenocalPrepared new chloramphenicol stocks
- Biofilm assay
    - stained wells of 24 hour plates with 125 µL of 0.1% crystal violet
        - dumped out crystal violet
        - rinsed in one beaker of water and tapped out crystal violet
        - rinsed in a second beaker of water and tapped dry onto a paper towel
    - no biofilms on the overnight 25ºC plate or the overnight 37ºC plate
- Used 15-15C and 15-16C to paint pictures on LB+Cam plates

Chloe/Charlotte

Cultures of *E. coli* with fluorescent protein genes were removed from the shaker. The new GFP culture looked promisingly green.

Plates from the freeze test were removed from the incubator. Growth was seen on all -20 plates, and several of the -80 plates. Increased growth on the stationary plates as opposed to the log-growth plates can likely be attributed to the much higher concentration of *E. coli* that was present on the stationary plates, as they were plated directly from the overnight culture. The 34C stationary plate from -80 seems to have a higher concentration of colonies on the 10-7 plate vs. the 10-5 plate; it's possible that the plates were switched during initial plating. The numbers have been swapped back in the table.

Colonies of Post-Freeze *E. coli* Plates								
Strain	Temp (°C)	Growth phase	Colonies from 10-5	Percent Survival	Colonies from 10-6	Percent Survival	Colonies from 10-7	Percent Survival
35C	-20	stationary	971	23.10%	493	17.46%	159	5.60%
35C	-20	log growth	335	59.08%	34	9.55%	13	6.07%
34C	-20	stationary	2008	102.65%	934	304.23%	238	626.32%
34C	-20	log growth	40	27.97%	15	5.88%	18	23.08%
RFP	-20	stationary	176	4.31%	37	1.66%	15	1.58%
RFP	-20	log growth	5	1.27%	2	1.45%	2	6.06%
35C	-80	stationary	6	0.14%	0	0%	0	0%
35C	-80	log growth	0	0%	0	0%	0	0%
34C	-80	stationary	84	4.29%	8	2.61%	2	5.26%
34C	-80	log growth	0	0%	1	0.39%	0	0%
RFP	-80	stationary	4	0.10%	1	0.04%	0	0%
RFP	-80	log growth	0	0%	0	0%	0	0%

We continued preparation for TouchTomorrow by painting with bacteria to make puzzles from square plates.

New glycerol stocks were prepared for 15-13C, 15-14C, 15-15C, and 15-16C; these were placed alongside the original glycerol stocks in the -80 freezer box. A glycerol stock for the bright GFP colony was also prepared.

Dave/Eddie

- We made 50ul of agarose gel to the above specifications, with 0.75g of gel powder.
- We ran another gel for the three samples of ZeAFP along with an uncut plasmid for a control. The concentration of the three samples was found as shown below:

Substance	Absorbance @260	Absorbance @280	Concentration (ug/ul)

15-4C 1	0.0049	0.0036	245
15-4C 2	0.0137	0.0115	685
15-4C 3	0.0183	0.0156	915

- Wells of the gel extract:
    - 1. ladder
    - 2. 15-4C 1
    - 3. 15-4C 1
    - 4. 15-4C 2
    - 5. 15-4C 2
    - 6. 15-4C 3
    - 7. 15-4C 3
    - 8. Uncut plasmid 15-4C
- The gel looked good, so separate extracts of each of the three samples were created with the following concentrations:

	Absorbance @260	Absorbance @2801	Concentration (ug/ul)
15-4C 1	0.0036	0.0024	180
15-4C 2	0.0073	0.0057	365
15-4C 3	0.0097	0.0074	485

- We performed a miniprep and created a glycerol stock of 15-17C according to protocol.
- The results of the miniprep

	Absorbance @260	Absorbance @280	Concentration (ug/ul)
15-17C 1	0.0161	0.0139	805
15-17C 2	0.0045	0.0037	225
15-17C 3	0.0057	0.0045	285

- The next step is to digest 15-17C with Spe and Pst then treat with CIP, then we will gel purify and ligate with the appropriate insert.
- Digest Table

Ingredients	15-17C (805 ug/ul)
DNA 5 ug	6.25 ul

Cutsmart 10x buffer	5 ul
Pst	2 ul
Spe	2 ul
qs to 50 ul	34.75 ul
Total	50 ul

- The digestion was run for 1 hour at 37C. CIP treatment involved the addition of CIP (1 ul) and incubation 20 min at 37C.
- The digest was then run on a gel with the following wells:
    - 1. 5 ul Ladder
    - 2. 26 u 15-17C
    - 3. 26 ul 15-17C
    - 4. 6 ul 15-17C uncut control
- A ligation was set up with 15-17C and 15-4C and left overnight:

Ingredient	**15-17C** + *15-4C*
Buffer (ul)	2
Ligase (ul)	1
Backbone (ul)	3
Insert (ul)	3
H2O	11
Total (ul)	20

- The transformations 15-8C with 15-5A and 15-6C worked, however neither of the 15-9C transformations succeeded, suggesting a problem with 15-9C. We repeated the ligation with varying amounts of 15-9C to determine if the problem is concentration. Only 15-5A was ligated, as it is needed for the interlab study while 15-6C was just being made for Touch Tomorrow. The ligation was set up as follows:

Ingredient	**15-9C** + *15-5A*	**15-9C** + *15-5A*	**15-9C** + *15-5A*
Buffer (ul)	2	2	2
Ligase (ul)	1	1	1
Backbone (ul)	1	2	5
Insert (ul)	3	3	3

H2O	13	12	9
Total (ul)	20	20	20

June 12, 2015

The whole team prepared the labs for Touch Tomorrow.

Dave/Eddie

- We conducted a transformation of the 15-17C/15-4C and the three 15-9C/15-5A samples
    - 2 ul of ligation was used for each transformation with 50 ul of competent cells
- We “painted” several more images using E. coli.
- The fluorescent E. coli was miniprepped according to protocol.

June 14, 2015

Dave/Eddie

- The plated transformation of 15-17C/15-4C showed no colony growth, and of the three 15-18C (15-9C/15-5A) samples, only the one with 5 ul of vector had colonies.
- Four colonies were picked from both the 15-18C plate and the 15-11C (15-8C/15-5A) plate from Thursday to grow overnight.